Clinical-Genetic Associations in the Prospective Huntington at Risk Observational Study (PHAROS) Implications for Clinical Trials

The Huntington Study Group PHAROS Investigators

**IMPORTANCE** Identifying measures that are associated with the cytosine-adenine-guanine (CAG) expansion in individuals before diagnosis of Huntington disease (HD) has implications for designing clinical trials.

**OBJECTIVE** To identify the earliest features associated with the motor diagnosis of HD in the Prospective Huntington at Risk Observational Study (PHAROS).

**DESIGN, SETTING, AND PARTICIPANTS** A prospective, multicenter, longitudinal cohort study was conducted at 43 US and Canadian Huntington Study Group research sites from July 9, 1999, through December 17, 2009. Participants included 983 unaffected adults at risk for HD who had chosen to remain unaware of their mutation status. Baseline comparability between CAG expansion (≥37 repeats) and nonexpansion (<37 repeats) groups was assessed. All participants and investigators were blinded to individual CAG analysis. A repeated-measures analysis adjusting for age and sex was used to assess the divergence of the linear trend between the expanded and nonexpanded groups. Data were analyzed from April 27, 2010, to September 3, 2013.

**EXPOSURE** Huntington disease mutation status in individuals with CAG expansion vs without CAG expansion.

**MAIN OUTCOMES AND MEASURES** Unified Huntington's Disease Rating Scale motor (score range, 0-124; higher scores indicate greater impairment), cognitive (symbol digits modality is the total number of correct responses in 90 seconds; lower scores indicate greater impairment), behavioral (score range, 0-176; higher scores indicate greater behavioral symptoms), and functional (Total Functional Capacity score range, 0-13; lower scores indicate reduced functional ability) domains were assessed at baseline and every 9 months up to a maximum of 10 years.

**RESULTS** Among the 983 research participants at risk for HD in the longitudinal cohort, 345 (35.1%) carried the CAG expansion and 638 (64.9%) did not. The mean (SD) duration of follow-up was 5.8 (3.0) years. At baseline, participants with expansions had more impaired motor (3.0 [4.2] vs 1.9 [2.8]; \( P < .001 \)), cognitive (\( P < .05 \) for all measures except Verbal Fluency, \( P = .52 \)), and behavioral domain scores (9.4 [11.4] vs 6.5 [8.5]; \( P < .001 \)) but not significantly different measures of functional capacity (12.9 [0.3] vs 13.0 [0.2]; \( P = .23 \)). With findings reported as mean slope (95% CI), in the longitudinal analyses, participants with CAG expansions showed significant worsening in motor (0.84 [0.73 to 0.95] vs 0.03 [−0.05 to 0.11]), cognitive (−0.54 [−0.67 to −0.40] vs 0.22 [0.12 to 0.32]), and functional (−0.08 [−0.09 to −0.06] vs −0.01 [−0.02 to 0]) measures compared with those without expansion (\( P < .001 \) for all); behavioral domain scores did not diverge significantly between groups.

**CONCLUSIONS AND RELEVANCE** Using these prospectively accrued clinical data, relatively large treatment effects would be required to mount a randomized, placebo-controlled clinical trial involving premanifest HD individuals who carry the CAG expansion.

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Huntington disease (HD) is typically an adult-onset, progressive, and fatal neurodegenerative disease characterized by movement disorder, cognitive decline, and behavioral disturbances. Huntington disease is an autosomal dominant condition caused by an expansion of a trinucleotide cytosine-adenine-guanine (CAG) in the 5′-translated first exon of the HTT gene on the short arm of chromosome 4. Individuals who inherit the CAG repeat expansion in the fully penetrant range (36-39) are associated with onset of clinical signs in some, but not all, individuals. Clinical onset of HD begins insidiously with abnormalities emerging gradually over many years during a premanifest or prodromal phase. Cognitive or behavioral abnormalities may antedate or coincide with motor abnormalities. Ultimately, specific extrapyramidal motor abnormalities emerge, leading to the clinical diagnosis of manifest HD.

A therapeutic goal of research in HD is the identification of treatments that delay the progression of disease and onset of illness in individuals at risk for developing manifest HD. Designing such efficacy trials is challenging. A major hurdle is the lack of practical primary outcome measures to assess the effect of an intervention on delaying disease onset. Use of the dichotomous end point of clinical diagnosis as the primary outcome requires large sample sizes and a long duration of follow-up to show a significant therapeutic effect on delaying disease onset. Continuous measures that can reliably distinguish individuals with CAG expansion in the premanifest period may allow for the identification of potential disease-modifying therapies using relatively smaller cohorts monitored for shorter periods. The need for such measures has become increasingly urgent since failed trials in other neurodegenerative diseases have prompted researchers to consider intervening in stages before significant pathologic insult has occurred.

In the Prospective Huntington at Risk Observational Study (PHAROS), raters blinded to HD mutation status assessed individuals at nominal 50% risk for inheriting the HD CAG expansion. Unlike other observational studies in this population (eg, Neurobiological Predictors of Huntington’s Disease [PREDICT-HD] and Track-HD), PHAROS represents the largest observational study to clinically evaluate premanifest HD wherein both research participants and investigators were unaware of HD mutation status. Accordingly, PHAROS was uniquely designed to address, in an unbiased manner, clinical features most associated with CAG expansion during the prodromal phase in HD. The identification of continuous outcome measures that are associated with HD in the premanifest period may facilitate the design and powering of future studies of potential disease-modifying therapies before traditional motor diagnosis.

Methods

Overview of PHAROS

PHAROS was designed to identify the earliest clinical features in adults at risk for HD who chose to remain unaware of their DNA CAG repeat status. From July 9, 1999, through December 31, 2004, a total of 1001 individuals were enrolled at 43 centers in North America. Participants were at nominal 50% risk for HD by virtue of having a clinically affected parent or sibling but had not met the criteria for a motor diagnosis of HD. Given that a proportion of previously at-risk individuals with CAG expansion would have already developed HD and not been eligible for PHAROS, the study was powered under the assumption that 60% of the participants were not carriers. At baseline, participants’ genomic DNA was genotyped for the CAG repeat size.

The institutional review boards at all participating sites approved the research protocols and consent procedures. All participants provided written informed consent and were not compensated for their involvement.

Clinical Assessments

Participants underwent motor, cognitive, psychiatric, and functional evaluations with the Unified Huntington’s Disease Rating Scale (UHDRS) at baseline and every 9 months thereafter as previously described. The motor section of the UHDRS was used to assess the presence and severity of motor features. Scores range from 0 to 124, and higher scores indicate greater impairment. Motor scores have been shown to be worse in prodromal HD participants when compared with control individuals without CAG expansion in PREDICT-HD, an observational study of individuals with prodromal HD and known HD mutation status. All motor assessments in PHAROS were performed by an independent rater experienced with HD and trained annually in the administration of the motor UHDRS. The independent rater completed only the motor assessment, which was used for the primary analysis of the motor outcomes.

The cognitive section of the UHDRS includes 3 cognitive tests: Symbol Digit Modalities (SDM), Verbal Fluency, and Stroop Interference. The SDM has shown sensitivity in prodromal HD. It was used as a representative cognitive task for purposes of the primary analysis and is scored on the number of correct responses in 90 seconds; cognitive tasks do not have a set maximum score. Verbal Fluency and Stroop Interference tasks were used as secondary cognitive outcomes since they have been demonstrated to be less sensitive in prodromal HD. For all cognitive tests, lower scores indicate greater impairment.

The behavior section of the UHDRS consists of 11 items separately evaluating the frequency and severity of the individual’s various behavioral symptoms. These scores are ranked on a 0 to 4 scale, with 0 indicating that the particular behavior is absent and 4 indicating that it is frequent or severe. Summing the product of frequency and severity items determines the total behavioral score (range, 0-176), with higher scores indicating greater behavioral symptoms.

The functional section of the UHDRS includes the Functional Assessment Check List, Independence Scale, and Total Functional Capacity (TFC). The TFC is a standard assessment of overall function in HD that demonstrates reliable progression in various manifest HD populations. The TFC is commonly used in manifest HD trials aimed at
showing an effect of an intervention on clinical progression. The TFC rates individuals’ function on the following domains: engagement in occupation, capacity to handle financial affairs, capacity to manage domestic chores, capacity to perform activities of daily living, and current living situation. An individual’s functional ability on each domain is summed, with scores ranging from 13 (normal function) to 0 (complete loss of function).

The UHDRS diagnostic confidence level was used to define motor diagnosis in at-risk individuals and is a probabilistic judgment based on the motor evaluation with a range from 0 (normal) to 4 (unequivocal signs of HD; ≥99% confidence on the part of the examiner). Participants were considered to have a motor diagnosis of HD when they first received a diagnostic confidence level score of 4 by the independent rater. The Box details the anchors of the scale. The diagnostic confidence level has previously shown fair interrater reliability (weighted $\kappa = 0.67$; SE, 0.09). For the primary analyses, all data from the participants, including data before and after a motor diagnosis, were used. All investigators and coordinators conducting the cognitive, behavioral, and functional assessments were experienced in the evaluation of HD and had been trained to conduct the UHDRS assessments.

**Genotype Assessment**

Coded venous blood samples from research participants were sent to the DNA laboratory of the Molecular Neurogenetics Unit at Massachusetts General Hospital. Genotyping of HTT (OMIM 613004) CAG was performed using a modification of the polymerase chain reaction amplification assay reported by Warner et al, with a fluorescent oligonucleotide primer pair flanking the repeat for automated allele calling (3730xl DNA Analyzer; Applied Biosystems) along with sequenced HD CAG allele standards as reported previously.

**Statistical Analysis**

**Analysis Cohort**

All participants enrolled in PHAROS were included in the analysis with the exception of 4 with missing CAG data and 14 who had a motor diagnosis (diagnostic confidence level, 4) at baseline. Participants with 37 or more CAG repeats were defined as carrying the expansion for HD (expanded), and those with less than 37 CAG repeats were defined as not carrying the expansion (nonexpanded). All available data, even those collected after a motor diagnosis was made, were included in the analyses except as noted.

**Baseline Comparisons**

Baseline comparability between the expanded and nonexpanded groups on demographic characteristics (age, sex, level of education, and affected parent) and on motor, cognitive, behavioral, and functional measures was assessed using Kruskal-Wallis tests, $\chi^2$ tests, or Fisher exact tests, as appropriate.

**Longitudinal Analyses**

For each clinical assessment, a repeated-measures analysis adjusting for age and sex was used to determine the divergence of the linear trend between the expanded and nonexpanded groups over time. This analysis was repeated in the expanded group, adjusting additionally for CAG as a continuous measure. Unlike the primary longitudinal analyses that included data on all participants before and after a motor diagnosis, a similar analysis, with participant data censored at the time of incident motor diagnosis, was performed to better understand the association of UHDRS measures in prodromal HD before the motor diagnosis.

**Item Analysis**

An item analysis was carried out to provide additional insight into the clinical characteristics and natural history in prodromal and early HD. Individual motor and behavioral items were dichotomized (score of 0 or normal vs score of ≥1 or abnormal) and analyzed by repeated-measures logistic regression to evaluate unique clinical motor and behavioral items (eg, chorea score and depression symptoms) that might be associated with CAG expansion during the premanifest period. To obtain convergence, age and sex were not included in these models, and the analysis period was limited to the first 10 visits.

**Sample Size Calculations**

To explore which outcomes would be most valuable in a clinical trial aimed at slowing HD progression in premanifest participants, we performed sample size calculations using changes in the total motor score, SDM, and TFC from baseline to the last visit as the primary outcome measures. Sample size calculations were for a randomized, placebo-controlled study in premanifest participants monitored for 1, 3, or 5 years. We assumed the data would be analyzed by a 2-sample $t$ test with a 2-sided $\alpha$ level of .05 and tabulated the sample sizes per group needed to give 80% power to detect reductions in the rate of decline of 50%, 40%, 30%, and 20% for each outcome measure.

The significance level was set at $P = .05$, and, because many of these analyses were exploratory, no adjustments were made for multiple comparisons. All analyses were conducted from April 27, 2010, to September 3, 2013, using SAS, version 9.2 (SAS Institute Inc).
Results

Analysis Cohort for Clinical-CAG Associations

Between 1999 and 2004, a total of 1001 participants were enrolled at 43 sites in North America. Of these, 983 participants met the criteria for the analysis (excluding 4 participants with missing CAG genotyped data and 14 participants who had an unequivocal motor diagnosis at baseline). In the group with expansions, 293 of 345 (84.9%) had diagnostic confidence level scores of 0 (normal) or 1 (nonspecific signs) compared with 607 of 638 (95.1%) of the nonexpanded group. Participants were monitored for a mean (SD) of 5.8 (3.0) years, with a range of 0 to 10 years (follow-up was completed December 17, 2009). A total of 163 participants (16.6%) completed 9 years of follow-up, and there was no differential drop-out rate between CAG expanded and nonexpanded individuals. The number of individuals by year of follow-up for the representative UHDRS domains is summarized in the Figure.

Of the analyzable cohort, 345 participants (35.1%) were found to carry the CAG expansion and 638 individuals (64.9%) did not carry the expansion. Baseline characteristics are reported in Table 1. At enrollment, participants with CAG expansions were younger than those without expansions (P = .02) but similar in sex, level of education, and HD-affected parent.

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>CAG &lt;37 Repeats (n = 638)</th>
<th>CAG ≥37 Repeats (n = 345)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>42.2 (7.3)</td>
<td>41.1 (7.2)</td>
<td>.02a</td>
<td></td>
</tr>
<tr>
<td>Educational level, y</td>
<td>14.8 (2.6)</td>
<td>15.0 (2.6)</td>
<td>.13a</td>
<td></td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>203 (31.8)</td>
<td>99 (28)</td>
<td>.31b</td>
<td></td>
</tr>
<tr>
<td>Affected father, No. (%)</td>
<td>278 (47.3)</td>
<td>142 (44.4)</td>
<td>.40b</td>
<td></td>
</tr>
<tr>
<td>Total motor score</td>
<td>1.9 (2.8)</td>
<td>3.0 (4.2)</td>
<td>&lt;.001a</td>
<td></td>
</tr>
<tr>
<td>Behavior score</td>
<td>6.5 (8.5)</td>
<td>9.4 (11.4)</td>
<td>&lt;.001a</td>
<td></td>
</tr>
<tr>
<td>Symbol digit score</td>
<td>53.6 (9.8)</td>
<td>52.3 (10.2)</td>
<td>.03a</td>
<td></td>
</tr>
<tr>
<td>TFC score</td>
<td>13.0 (0.2)</td>
<td>12.9 (0.3)</td>
<td>.23a</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CAG, cytosine-adenine-guanine; TFC, Total Functional Capacity.

a Kruskal-Wallis tests.

b Fisher exact test or χ² test.

c Not all participants provided information on the affected father (CAG <37 repeats, 588; and CAG ≥37 repeats, 320).

d CAG expansions were younger than those without expansions (P = .02) but similar in sex, level of education, and HD-affected parent.
UHDRS Outcomes by Mutation Status

At baseline, UHDRS scores for total motor (P < .001), SDM (P = .03), and behavior (P < .001) were worse for individuals with CAG expansions compared with those without expansions, but there were no functional differences as measured by the TFC (Table 1) or other functional measures as measured by the mean (SD) (25.0 [0.29] vs 25.0 [0.15] for the Functional Assessment Check List and 100.0 [0.81] vs 100.0 [0.40] for the Independence Scale). Verbal Fluency did not distinguish expanded from nonexpanded participants at baseline (P = .52), but Stroop Interference differed significantly (P = .02). The Figure illustrates the change over time in UHDRS scores for total motor, SDM, behavior, and TFC by the groups with and without CAG expansions.

Table 2 summarizes the mean slope (change per year) for the UHDRS scores for total motor, SDM, behavior, and TFC for the entire cohort and for the analyses that censored participants’ data at the time of motor diagnosis. Total motor, SDM, and TFC scores showed significant worsening over time in the expanded group compared with the nonexpanded group. Although the expanded group’s behavioral scores were worse at baseline, they did not significantly diverge over time compared with the nonexpanded group. Both Verbal Fluency and Stroop Interference tests showed significant worsening over time in the expanded group compared with the nonexpanded group (P < .001 for both). Inclusion of quadratic terms in the models did not substantially affect the conclusions of these analyses, which suggested that the change was linear. Results for the expanded group were similar when analyses were also adjusted for CAG repeat length.

Analysis results for each group demonstrated that total motor scores worsened over time (P < .001) in the group with CAG expansions, but there was no significant change in the group without expansions (P = .45). Symbol Digit Modalities worsened in the group with expansions (P < .001) but improved in the group without expansions (P < .001). Behavioral scores improved in both groups (P = .01 with and P < .01 without expansions). Total Functional Capacity worsened in the group with expansions (P < .001), with no significant change in the group without expansions (P = .26).

When data from participants given a motor diagnosis of HD during longitudinal follow-up (n = 61) were excluded starting at the time of the motor diagnosis, the baseline differences and divergence in UHDRS scores were less pronounced but similar to those in the primary analysis (eFigure in the Supplement). Again, there was significant worsening in total motor scores, SDM, and TFC in the group with expansions compared with the group without expansions but no divergence in behavioral scores (Table 2).

Analysis of the individual motor items demonstrated significant abnormalities in chorea, voluntary motor tasks, speech, and the Luria maneuver at baseline that worsened over time in the group with expansions compared with the group without expansions. Oculomotor, dystonia, and tongue protrusion items were not significantly different at baseline between the groups, but these items worsened over time in the group with expansions compared with the group without expansions. Depressed mood and irritability were the only individual behavioral items that distinguished the groups at baseline, suggesting that depressive symptoms may account for the difference seen at baseline in the behavioral domain. Nevertheless, no behavioral items worsened over time in either group. The eTable in the Supplement provides detailed data on the individual motor and behavioral item analysis.

Sample Size Considerations

Table 3 reports the sample size estimates based on the PHAROS data for premanifest participants carrying the CAG expansion enrolled in a placebo-controlled trial of a treatment with a 50%, 40%, 30%, and 20% reduction in the rate of decline on total motor, SDM, and TFC monitored for 1, 3, and 5 years. In all cases, treatments with small effects require unrealistically large numbers of participants. Total motor scores with a relatively robust 50% treatment effect appear to be the most powerful measure with sample sizes of 352 to 217 participants per group for a 1-year and 5-year study, respectively.

Discussion

To our knowledge, PHAROS is the largest observational study to prospectively evaluate premanifest HD wherein both participants and raters were unaware of mutation status. It therefore represents an optimal cohort to address the association of clinical features and mutation status in prodromal HD prior to unequivocal motor manifestations of HD. In PHAROS, the CAG-expanded participants, compared with the CAG-nonexpanded ones, showed subtle motor, cognitive, and behavioral features at baseline, with motor, cognitive, and functional measures worsening over time. Even

Table 2. The Mean Slope of Representative UHDRS Domainsa

<table>
<thead>
<tr>
<th>Domain</th>
<th>All Participants</th>
<th>Participants Censoredb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAG &lt;37 Repeats</td>
<td>CAG ≥37 Repeats</td>
</tr>
<tr>
<td></td>
<td>CAG &lt;37 Repeats</td>
<td>CAG ≥37 Repeats</td>
</tr>
<tr>
<td>Total motor score</td>
<td>0.03 (−0.05 to 0.11)</td>
<td>0.84 (0.73 to 0.95)</td>
</tr>
<tr>
<td>Symbol Digit Modalities</td>
<td>0.22 (0.12 to 0.32)</td>
<td>−0.54 (−0.67 to −0.40)</td>
</tr>
<tr>
<td>Behavior</td>
<td>−0.27 (−0.39 to −0.16)</td>
<td>−0.22 (−0.38 to −0.06)</td>
</tr>
<tr>
<td>TFC</td>
<td>−0.01 (−0.02 to 0)</td>
<td>−0.08 (−0.09 to −0.06)</td>
</tr>
</tbody>
</table>

Abbreviations: CAG, cytosine-adenine-guanine; TFC, Total Functional Capacity; UHDRS, Unified Huntington’s Disease Rating Scale.

a Change per year, reported as mean (95% CI).

b Data from individuals with a motor diagnosis (phenoconverters) were censored from further analyses at the time of motor diagnosis.

c Using the repeated-measures analysis and adjusting for age and sex.

d Sum of the product of frequency and severity items.
when excluding data after a participant was given a motor diagnosis, the distinctions persisted, indicating that they are not merely the result of development of abnormalities near the time of motor phenoconversion. Taken together, these findings suggest that abnormalities detected on the UHDRS are associated with CAG expansion in premanifest HD and may serve as distinctive outcomes in trials aimed at slowing the clinical decline or forestalling the onset of illness in this population.

The presence of subtle motor, cognitive, and behavioral abnormalities in prodromal HD is now well recognized, and data from other premanifest cohorts also show that striatal atrophy and clinical features may begin to emerge even decades before the motor diagnosis. PHAROS differs from the other cohorts in that all data were collected with CAG status concealed from both participants and raters, reducing bias in scoring and further confirming the genetic association of characteristic HD clinical features in the relatively lengthy prodromal period before the development of sufficient motor abnormalities to support a motor diagnosis of HD. Furthermore, the independent rater scores used as the primary motor score outcome were not biased by rater knowledge of other potential HD symptoms or participant data. Notably, total motor scores were the only item that worsened substantially in the expanded group, remaining essentially unchanged in the nonexpanded group. This finding suggests that differences seen in motor worsening during the premanifest period are associated with CAG expansion.

As anticipated, motor features of chorea and impaired fine motor control were more commonly affected early and worsened over time in the group with CAG expansions compared with their nonexpanded control counterparts. These findings are consistent with results from the large PREDICT-HD cohort, which primarily examined clinically unaffected individuals with CAG expansions, and in smaller studies showing that subtle hyperkinesia and impaired coordination distinguish mutation carriers from controls. However, unlike previous studies, we were unable to show differences at baseline in measures of ocular motility, which is commonly believed to be one of the earliest motor features of disease. This result may have occurred because our cohort could have been slightly further from onset than prior cohorts or that our “bedside” clinical ratings were not sufficiently sensitive to detect subtle abnormalities in eye movements. The former explanation is supported by the fact that ocular motility measures worsened over time and by an additional analysis of motor scores by estimated probability of onset suggesting that participants who were further from onset looked more like nonexpanded than other expanded individuals. Ultimately, this analysis confirms earlier findings of motor abnormalities in prodromal HD and suggests that some features, such as chorea and impaired coordination, may occur earlier and be useful for tracking progression in prodromal HD.

Likewise, certain cognitive measures are impaired early and worsen over time in the CAG-expanded compared with the CAG-nonexpanded participants. Results from both SDM and Stroop Interference were associated with CAG expansion at baseline in premanifest HD. Although Verbal Fluency did not adequately distinguish expanded from nonexpanded participants at risk for HD at baseline, it worsened over time in the CAG-expanded cohort. Verbal Fluency has been shown to be variably affected in premanifest HD and may reflect more generalized cognitive impairment; therefore, it may not be sensitive at this early stage of the disease. In addition, some of the divergence on cognitive outcomes between expanded and nonexpanded participants may be partially accounted for by improvements (presumably reflecting practice effects) in the nonexpanded group. This finding is consistent with previous data suggesting a lack of practice effects in premanifest HD. Regardless, our results confirm the presence of early and meaningful cognitive effects in this cohort and are consistent with PREDICT-HD findings, suggesting the value of SDM as a potential cognitive outcome for use in clinical trials in premanifest HD.

Behavioral features also distinguished CAG-expanded from CAG-nonexpanded participants at baseline but, surprisingly, without further divergence between the groups over time. Differences in behavior measures were largely accounted for by symptoms associated with depression. Other reports have similarly indicated that behavioral abnormalities remain constant from the premanifest stage onward. This finding suggests that behavioral manifestations in individuals at risk for HD are not necessarily a consequence of living within an HD-affected family but could be related to genetic influences associated with the CAG expansion or other features of HD, such as cognitive impairment. Behavioral scores improved in both groups over time, perhaps related to the salutary effects of being enrolled in a long-term study, including the access provided for recognition and treatment of impaired mental health. However, HD observational studies and clinical trials usually exclude individuals from enrolling who have prominent behavioral disturbances that may interfere with their research participation.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)</th>
<th>Slowing of Rate of Decline, No. 50% 40% 30% 20%</th>
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<tbody>
<tr>
<td>Motor</td>
<td></td>
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</tr>
<tr>
<td>1 y</td>
<td>1.80 (4.26)</td>
<td>352</td>
</tr>
<tr>
<td>3 y</td>
<td>2.52 (5.63)</td>
<td>314</td>
</tr>
<tr>
<td>5 y</td>
<td>4.03 (7.49)</td>
<td>217</td>
</tr>
<tr>
<td>SDM test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 y</td>
<td>3.06 (8.54)</td>
<td>490</td>
</tr>
<tr>
<td>3 y</td>
<td>2.83 (9.57)</td>
<td>719</td>
</tr>
<tr>
<td>5 y</td>
<td>4.54 (9.47)</td>
<td>273</td>
</tr>
<tr>
<td>TFC test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 y</td>
<td>−0.07 (0.41)</td>
<td>2209</td>
</tr>
<tr>
<td>3 y</td>
<td>−0.06 (0.62)</td>
<td>7647</td>
</tr>
<tr>
<td>5 y</td>
<td>−0.17 (0.72)</td>
<td>1138</td>
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</table>

Abbreviations: SDM, Symbol Digit Modalities; TFC, Total Functional Capacity. *For SDM, the mean change in the group with cytosine-adenine-guanine expansion was subtracted from the mean change in the group without expansion to eliminate the learning effect.
A major objective of PHAROS was the identification of clinical features in premanifest participants that worsen over time and that may be relevant and useful as clinical outcome measures in long-term trials at modifying the course of HD. In PHAROS, motor scores, followed by cognitive measures, appeared to be the most specific for CAG expansion and worsened over time during the premanifest period. In contrast, participant-reported behavioral measures improved and TFC declined marginally. In fact, TFC worsened in both groups, although statistically significantly only in the expanded group, and at a significantly greater rate in the expanded compared with the nonexpanded group, suggesting that functional capacity is minimally affected during the prodromal period. Unlike HD trials in manifest patients that use TFC, our data suggest that such functional measures designed to detect disability would be a relatively uninformative outcome in trials enrolling premanifest participants. Therefore, functional outcomes would need to be revised to measure functional domains that are sensitive to decline in the prodromal period. In addition, our calculations indicate that analyses of cognitive and functional outcomes would require very large sample sizes that would not likely be feasible, even assuming very large effect sizes. Total motor scores, which require the smallest sample sizes, would still require large effect sizes, suggesting that clinically relevant outcomes that are highly sensitive to change and of low variability are still needed. Alternatively, study designs that enrich cohorts for individuals with factors such as being closer to onset and having higher CAG-product scores may be needed to enhance the feasibility of trials in this population.

Our study and analyses have several limitations. Although the population was selected from at-risk individuals who chose not to undergo DNA predictive testing, there may have been a selection bias for mutation-positive individuals who chose not to undergo DNA predictive testing, theremay though the population was selected from at-risk individuals who have been a selection bias for mutation-positive individuals who chose not to undergo DNA predictive testing, therefore, functional outcomes would need to be revised to measure functional domains that are sensitive to change and of low variability are still needed. Alternatively, study designs that enrich cohorts for individuals with factors such as being closer to onset and having higher CAG-product scores may be needed to enhance the feasibility of trials in this population.

Our study and analyses have several limitations. Although the population was selected from at-risk individuals who chose not to undergo DNA predictive testing, there may have been a selection bias for mutation-positive individuals with relatively greater clinical manifestations, including subtype, motor, cognitive, and behavioral abnormalities at baseline. However, the study was blinded to mutation status and, because most participants had normal or nonspecific results of examinations, this was unlikely to have a significant effect on concealment. There was a marked sex disparity in our cohort, with twice as many women than men enrolled. Therefore, our cohort may not be an accurate representation of therapeutic trials in which there is a more even distribution of the sexes. An even distribution may be important because sex may influence response to therapeutic interventions as well as HD phenotype through gene–sex interactions. However, we did not find gene–sex interactions in our cohort. In addition, we did not identify large discrepancies in behavioral or functional measures; however, we did not consistently use family informants to ascertain behavior or function. Data from PREDICT-HD suggest that there may be a divergence in symptom reporting between participants and informants as individuals approach the time of diagnosis. Finally, our cohort may not be representative of participants enrolling in future premanifest HD clinical trials who are likely to have already undergone genetic testing. However, our findings are consistent with those from PREDICT-HD, in which at-risk participants had already undergone genetic testing. Despite these limitations, PHAROS was able to demonstrate the clinical features associated with CAG expansion under conditions wherein both participants and investigators were unaware of CAG mutation status.

Conclusions

In a population before HD motor diagnosis, there are significant baseline motor, cognitive, and behavioral differences between individuals with and without CAG expansions. Over time, motor and cognitive measures in this premanifest population worsen. The ability of these measures to distinguish individuals with CAG expansion from nonexpanded controls suggests that these variables could be used as outcome measures in clinical trials aimed at slowing disease progression or delaying disease onset in premanifest HD. However, the large sample sizes required to detect meaningful changes may not be feasible or justifiable. Still needed are relevant outcomes, clinical or biological, that are highly sensitive to change and of low variability among clinically unaffected individuals who have inherited the HD mutation.

ARTICLE INFORMATION
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REFERENCES


